Use of autologous bone marrow mononuclear cell implantation therapy as a limb salvage procedure in patients with severe peripheral arterial disease

Randall W. Franz, MD, FACS, RVT,[a] Alan Parks, DO,[b] Kaushal J. Shah, MD, RPVI,[a] Thomas Hankins, CCP,[c] Jodi F. Hartman, MS,[d] and **Michelle L. Wright, MPH,[d]** *Columbus and Gahanna, Ohio*

Background: **Few options other than amputation exist for some patients with peripheral arterial occlusive disease (PAD) and severe anatomical limitations.**

Methods: **This prospective study presents short-term results of dual intramuscular and intra-arterial autologous bone marrow mononuclear cell (BM-MNC) implantation for the treatment of patients with severe PAD in whom amputation was considered the only viable treatment option. Baseline, two-week, and three-month evaluations were conducted. Ankle brachial indices (ABI) were calculated for both the dorsal pedis and the posterior tibial arteries. Rest pain and ulcer healing also were assessed. Success was defined as meeting the following four criteria: improvement in ABI measurements; relief of rest pain; ulcer healing, if applicable; and absence of major limb amputations. Patients not undergoing major limb amputations continued to be monitored for subsequent procedures.**

Results: **Nine patients for whom limb amputation was recommended underwent this procedure. The study population was comprised of five females and four males, with a mean age of 61.7 years. Eight (88.9%) patients had rest pain. Seven (77.8%) patients also had diabetes. Non-healing ulcers were present in eight (88.9%) cases. After the procedure, non-significant improvements of 0.12 and 0.08 in ABI were observed for the dorsalis pedis and posterior tibial ankle arteries, respectively. Three (33.3%) major amputations subsequently were performed, including a below-knee amputation 4.1 weeks after the BM-MNC implantation and two above-knee amputations at 5.4 and 11.0 weeks after the procedure. The six (66.7%) patients who did not have major amputations demonstrated improvement in symptom severity three months after the procedure, as evidenced by alleviation of rest pain and improvements by at least one level in Rutherford and Fontaine classifications, and have not required amputations at a mean follow-up of 7.8 months. Complete wound healing was achieved within three months in all patients who had ulcers prior to BM-MNC implantation and for whom amputation was not required. This specific BM-MNC implantation technique was fully successful in three (33.3%) patients, as major amputation was avoided and the other applicable criteria were met. Five (55.6%) additional patients demonstrated success in at least one of the four criteria.**

Conclusions: **With eight (88.9%) of nine patients showing some level of improvement and amputation avoided in six (66.7%) patients, these short-term results indicate the use of BM-MNC implantation as a means of limb salvage therapy for patients with severe PAD shows promise in postponing or avoiding amputation in a patient population currently presented with few alternatives to amputation. (J Vasc Surg 2009;50:1378-90.)**

The prevalence of peripheral arterial occlusive disease (PAD) has been increasing in recent years, affecting a reported eight to 12 million people in the United States.[1,2] Risk factors associated with the development of PAD include individuals aged 65 years and older, nicotine use, diabetes mellitus, hypertension, hypercholesterolemia, and family history of cardiovascular disease.[1,3,4] Although patients with PAD may be asymptomatic, intermittent claudication (leg pain after walking) is the most common presenting symptom.[2-4] As PAD progresses, leg pain at rest and/or ischemic ulceration often are hallmark signs of ischemia.[5,6]

Treatment for PAD is dependent on the severity of the symptoms and typically ranges from conservative pharmaceutical management with risk factor modifications to endovascular or open surgical interventions in more severe cases.[2,6] Patients with end-stage PAD often have multilevel disease or are unable to be treated with these traditional methods, leaving major limb amputation as the only treatment option.[2,6] Over 100,000 limb amputations are performed each year due to PAD.[5] Furthermore, the annual cost associated with amputations in the United States is an estimated $13 billion.[7,8]

In the past 10 years, therapeutic angiogenesis using bone marrow mononuclear cells (BM-MNC) has become a promising treatment for patients with moderate to severe PAD who have no other treatment options.[6,9] The goal of treatment is to promote neoangiogenesis (new vessel formation) in ischemic tissues.[10] Bone marrow cells possess various types of primitive cells that are able to differentiate

From the Vascular and Vein Center at Grant Medical Center,[a] the Doctors Hospital,[b] the Grant Medical Center,[c] and Orthopaedic Research and Reporting, Ltd.[d]

Competition of interest: none.

Reprint requests: Randall W. Franz, MD, FACS, RVT, The Vascular and Vein Center at Grant Medical Center, 285 East State St., Ste. 260, Columbus, OH 43215 (e-mail: rfranz2@ohiohealth.com).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a competition of interest.

0741-5214/$36.00

doi:10.1016/j.jvs.2009.07.113

Table I. Demographics, limb involvement, and ischemia status listed by patient

Patient number	*Age (years)*	*Gender*	*Smoking status*	*Ulcer status*			*Rutherford classification*		*Fontaine classification*
				Presence of Non-healing ulcers (Y or N)	*If Y, ulcer location*	*Presence of gangrene (Y or N)*	*Grade*	*Category*	
1	58	Male	Smoker	Y	First toe	Y	III	6	IV
2	73	Female	Smoker	N	—	N	II	4	III
3	85	Male	Non-smoker	Y	Foot	Y	III	5	IV
4	43	Female	Smoker	Y	Anterior tibia	N	III	5	III
5	75	Female	Smoker	Y	First, second, and fourth toes	Y	III	6	IV
6	54	Female	Smoker	Y	Lower extremity and foot	N	III	5	IV
7	57	Female	Smoker	Y	Foot	N	III	5	IV
8	60	Male	Smoker	Y	Foot	Y	III	5	IV
9	55	Male	Smoker	Y	First toe	N	III	5	IV

Table II. Co-morbidities and prior vascular procedures listed by patient

Patient number	*Cerebrovascular accident*	*Chronic obstructive pulmonary disease*	*Coronary artery disease*	*Diabetes*	*Hyperlipidemia*	*Hypertension*	*Other*	*Prior vascular procedure*	
								Major open	*Endovascular*
1	Absent	Absent	Absent	Present	Present	Present	—	Yes	Yes
2	Present	Absent	Present	Absent	Present	Present	—	Yes	No
3	Absent	Absent	Absent	Present	Absent	Present	—	No	Yes
4	Present	Absent	Present	Present	Absent	Present	Seizure	Yes	Yes
5	Absent	Present	Absent	Absent	Absent	Present	—	No	Yes
6	Absent	Absent	Absent	Present	Present	Present	Hypothyroid	Yes	Yes
7	Present	Present	Present	Present	Present	Present	—	Yes	Yes
8	Present	Present	Present	Present	Present	Present	ESRD, HIT	Yes	No
9	Absent	Absent	Absent	Present	Absent	Present	ESRD	No	No

ESRD, End-stage renal disease; *HIT,* heparin-induced thrombocytopenia.

Table III. Dorsalis pedis artery and posterior tibial artery ABI measurements, rest pain, and ischemic ulceration status before and after BM-MNC implantation and current amputation status listed by patient

Patient number	*Ankle brachial index (ABI)*						*Rest pain*		*Ischemic ulceration*		*Major amputation Post-BM-MNC (Y or N)*	*If yes, type of major amputation*
	Dorsalis pedis artery			*Posterior tibial artery*								
	Pre	*Post*	*Change (Post–Pre)*	*Pre*	*Post*	*Change (Post–Pre)*	*Pre*	*Post*	*Pre*	*Post*		
1	0.00	0.00	0.00	0.00	0.00	0.00	Present	—	Present	—	Y	Above knee
2	0.45	0.47	0.02	0.40	0.46	0.06	Present	Absent	Absent	—	N	—
3	0.50	0.53	0.03	0.48	0.65	0.17	Present	Absent	Present	Present	N	—
4	0.43	0.39	−0.04	0.47	0.44	−0.03	Present	Absent	Present	Absent	N	—
5	0.29	0.44	0.15	0.28	0.35	0.07	Present	—	Present	—	Y	Below knee
6	0.00	0.00	0.00	0.00	0.22	0.22	Present	—	Present	—	Y	Above knee
7	0.00	0.16	0.16	0.00	0.00	0.00	—	—	Present	Absent	N	—
8	0.00	0.74	0.74	0.47	0.69	0.22	Present	Absent	Present	Absent	N	—
9	NC	NC	—	NC	NC	—	Present	Absent	Present	Present	N	—

ABI, Ankle brachial index; *BM-MNC,* bone marrow mononuclear cell; *NC,* non-compressible.

into hematopoietic and mesenchymal cells and secrete growth factors that promote neoangiogenesis and endothelialization.[10-14] Previously published animal and clinical studies have demonstrated that implantation of autologous BM-MNC is a safe and effective technique in the development of collateral arteries.[2,6,9-12,14-22] In this study, dual intramuscular and intra-arterial autologous BM-MNC implantation was performed in patients with severe PAD in

Table IV. Comparison of dorsalis pedis artery and posterior tibial artery ABI measurements before and after BM-MNC implantation

	Dorsalis pedis artery ABI		*Posterior tibial artery ABI*	
	Pre-implantation n = 8	*Post-implantation n = 8*	*Pre-implantation n = 8*	*Post-implantation n = 8*
Mean ± SD	0.21 ± 0.23	0.34 ± 0.26	0.26 ± 0.23	0.31 ± 0.27
Median	0.15	0.42	0.34	0.40
Range (min-max)	0.00-0.50	0.00-0.74	0.00-0.48	0.00-0.69

ABI, Ankle brachial index; *BM-MNC,* bone marrow mononuclear cell; *SD,* standard deviation.

whom amputation was considered as the only viable treatment option with the goal of improving tissue perfusion to salvage the limb. The authors hypothesized that patients undergoing autologous BM-MNC implantation would experience improved ankle-brachial index (ABI) measurements, relief of rest pain, and ulcer healing, when applicable, compared with pre-procedure evaluation and would not require major limb amputation.

METHODS

This prospective study was initiated with institutional review board approval and was designed to evaluate the outcomes of autologous BM-MNC implantation for the treatment of severe PAD. All procedures were performed by one of the senior authors using standardized interventional techniques. Between December 2007 and January 2009, nine patients provided written informed consent and were enrolled in the study. Patients at least 18 years of age were eligible for study participation if they had severe limb-threatening PAD, defined as ABI less than 0.4 or presence of non-healing ischemic ulcers; and stenosis or occlusion of two of the following lower extremity arteries: anterior tibial, posterior tibial, and peroneal. Additional stenosis or occlusion may be present proximally to these vessels. Major amputation was recommended for these patients due to limb-threatening ischemia or the presence of ulcers that could not be healed by conservative wound management. Patients with conditions that precluded recommendation of traditional endovascular or open bypass treatments were eligible for inclusion. Patients younger than 18 years of age and those eligible to undergo traditional endovascular or open bypass for the treatment of PAD were excluded from study participation. Pregnant women, prisoners, patients with mental retardation, and those unable to consent for participation independently also were excluded. At the time of enrollment, all patients were receiving maximum medical therapy for PAD at the discretion of the managing vascular surgeon and wound care management, when applicable.

Study design. Patients with ulcers received extensive wound care management per routine protocols at the wound care clinic associated with our institution prior to and after BM-MNC implantation. Evaluations were conducted prior to BM-MNC implantation and at two weeks and three months after the procedure. The extent of acute ischemia for the involved limb was classified using the Rutherford criteria, whereas the Fontaine classification was used to classify PAD.[23,24] The two-week post-procedure assessment was conducted to determine the presence of procedure-related complications only and no study data were collected. Rest pain and ulcer status, if applicable, were assessed at the pre-treatment and three-month post-treatment evaluations.

Table V. Outcomes of BM-MNC implantation using four-pronged definition of success

	N = 9
Improvement in all four criteria for ulcer patients, or in all three applicable criteria for non-ulcer patients	3 (33.3%)
• Improvement in ABI measurement(s), absence of rest pain, ulcer healing, and no major limb amputations	1 (11.1%)
• Improvement in ABI measurement(s), absence of rest pain, and no major limb amputations	1 (11.1%)
• Improvement in ABI measurement(s), ulcer healing, and no major limb amputations	1 (11.1%)
Improvement in three of the four criteria	2 (22.2%)
• Improvement in ABI measurement(s), absence of rest pain, and no major amputations	1 (11.1%)
• Absence of rest pain, ulcer healing, and no major amputations	1 (11.1%)
Improvement in at least two of the four criteria	1 (11.1%)
• Absence of rest pain and no major amputations	1 (11.1%)
Improvement in at least one of the four criteria	2 (22.2%)
• Improvement in ABI measurement(s)	2 (22.2%)
No improvement in any criteria	1 (11.1%)

ABI, Ankle brachial index; *BM-MNC,* bone marrow mononuclear cell.

Routine angiography was performed prior to the bone marrow harvest to identify the specific location of stenosis or occlusion. When possible, Doppler-guided arterial segmental pressures of the dorsal pedis artery and posterior tibial artery were measured prior to the procedure and three months after BM-MNC implantation. Ankle brachial indices were calculated separately for each of the lower extremity arteries by dividing the ankle systolic pressure of the individual artery by the brachial artery systolic pressure. Finally, amputations also were monitored and categorized into minor and major limb amputations. Individual toe and transmetatarsal (TMT) amputations were considered minor, whereas below- and above-knee amputa-

tions were classified as major amputations. Patients not undergoing major limb amputations continued to be monitored for subsequent procedures. Success of autologous BM-MNC implantation was defined as meeting the following four criteria: improvement of ABI measurements; relief of rest pain; ulcer healing, if applicable; and absence of major limb amputations.

Statistical analysis. Statistical analysis was performed using SigmaStat Software, version 2.0 (SPSS, Inc, Chicago, Ill). Descriptive statistics, including mean, median, standard deviation, frequency, and percentage were used to describe demographic and clinical data. Ankle brachial index measurements were compared between the pre- and post-procedure time intervals, as possible. Paired *t* test was performed to compare normally distributed continuous variables, whereas the Wilcoxon signed rank test was employed to compare non-normally distributed variables. Ninety-five percent confidence intervals were used throughout the statistical analysis. Statistical differences were considered significant when the *P*-value was less than or equal to 0.05 with a power of at least 0.8.

Bone marrow harvest and isolation of mononuclear cells. While patients were under minimum alveolar concentration (MAC) and local anesthesia, autologous bone marrow aspirate was obtained from the anterior superior iliac spine (ASIS), which was prepped and draped prior to surgery. A #11 blade was utilized to create a 2-mm incision anterior to the ASIS. A three-hole bone marrow needle (DePuy, Inc., Warsaw, Indiana, USA) with a syringe containing anticoagulant (heparin, 1,000 units/mL) was utilized for the bone marrow aspiration. For a patient with heparin-induced thrombocytopenia, only saline was used in the syringe. The initial trajectory of the needle was medially in line with the pelvic wing and aimed slightly posterior to the iliac tubercle to allow entry into the medullary canal directly beneath it. The needle tip was advanced through the cortex by gently tapping the needle with a mallet. A total of 50 mL to 60 mL of aspirate was obtained. The aspirate was placed in a centrifuge and was spun at 2400 rpm for twelve minutes to concentrate the bone marrow cells (GenesisCS Component Concentrating System; EmCyte Corporation, Fort Myers, Fla).

Autologous BM-MNC implantation. The concentrated cells obtained after centrifugation were placed in a sterile container. The cells were immediately reinjected intramuscularly and intravascularly into the patient using injection sites determined by location of stenosis and/or occlusion on angiogram obtained prior to implantation. For the intramuscular injections, 2 mL of aspirate were injected intramuscularly into six different lower leg sites in a standardized fashion to approximate the disease surface area. Intramuscular injections specific to the calf started 8 cm above the medial and lateral malleolus in the midline of the calf and continued proximally in 8-cm increments. Intramuscular injections in the thigh began 8 cm above the knee joint, both medially and laterally, and continued proximally in 8-cm increments. For the intra-arterial injection, 12 mL of aspirate was injected intravascularly in a 4 French catheter in 2 mL increments, with flushing between injections. Occlusion location determined artery selection, with injections occurring in the popliteal artery for tibial occlusions and in the common femoral artery for superficial femoral artery occlusions.

Study population. Patient demographics, ulcer status, and the Rutherford and Fontaine Classifications are presented in Table I. The population was comprised of five (55.3%) females and four (44.4%) males and included six (66.7%) African-American and three (33.3%) white patients. The mean age of the study population was 61.7 years (SD = 13.4; median = 58.0; range, 43.0-85.0 years). Eight (88.9%) patients were smokers. Six (66.7%) cases involved the left and three (33.3%) cases involved the right lower extremity. Aside from one patient for whom rest pain could not be assessed due to hemiparesis from a previous cerebrovascular accident (CVA), all had rest pain prior to BM-MNC implantation. The remaining patient had a non-healing foot ulcer, but rest pain could not be assessed due to hemiparesis occurring after a CVA prior to study enrollment. Non-healing ulcers were present in eight (88.9%) cases; six (75.0%) were on the foot and two (25.0%) were lower extremity ulcers. Gangrene was present in four (50.0%) of the eight cases with ulcers. Baseline Rutherford classifications included one (11.1%) II4 (ischemic rest pain); six (66.7%) III5 (minor tissue loss – nonhealing ulcer, focal gangrene with diffuse pedal ischemia); and two (22.2%) III6 (major tissue loss, extending above transmetatarsal level, functional foot no longer salvageable). Two (22.2%) patients had Fontaine class III PAD, corresponding to rest pain, and seven (77.8%) patients had Fontaine class IV PAD, corresponding to pedal necrosis.

Co-morbidities and prior vascular procedures are listed on a per-patient basis in Table II. The co-morbidity distribution for the study population was as follows: nine (100.0%) hypertension; seven (77.8%) diabetes; five (55.6%) hyperlipidemia; four (44.4%) CVAs; four (44.4%) coronary artery disease; three (33.3%) chronic obstructive pulmonary disease; two (22.2%) renal disease; one (11.1%) heparin-induced thrombocytopenia; one (11.1%) hypothyroidism; and one (11.1%) seizure history. Five (55.6%) patients had previous endovascular procedures. Five (55.6%) patients had prior major open procedures, which included bypasses and minor amputations.

RESULTS

No procedure-related complications were reported during the technique or at the two-week and three-month follow-up evaluations. Table III summarizes ABI measurements, rest pain, and ischemic ulceration status obtained before and after BM-MNC implantation and also lists the current amputation status for the nine study patients. The ABI measurements obtained after BM-MNC implantation are compared with the pre-implantation measurements in Table IV. Although improvement in ABI measurements was observed after BM-MNC implantation for both the dorsalis pedis (mean = 0.13, SD = 0.26, median = 0.03, range = −0.4-0.74) and the posterior tibial (mean = 0.09,

Table VI. Comparison of study type, procedure indications, and patient demographics between previously published studies utilizing BM-MNC for the treatment of lower extremity ischemia

	Study type	*Country*	*Indication(s)*
Intra-arterial and intramuscular BM-MNC implantation			
Current Study 2009	Prospective, case series	USA	Severe PAD (Amputation recommended) (Rutherford II or III; Fontaine III or IV)
Van Tongeren et al, J Cardiovasc Surg 2008[32]	Prospective, non-randomized	Netherlands	PAD (no options for revascularization) (CLI or claudication)
Bartsch et al, Clin Res Cardiol 2007[15]	Prospective, open label, controlled	Germany	Long-Lasting Severe PAD (Fontaine IIB) (including 3 TAO)
Intra-arterial BM-MNC Implantation			
Chochola et al, Int Angiol 2008[26]	Prospective, case series	Czech Republic	CLI (Fontaine III or IV)
Cobellis et al, Bone Marrow Transplant 2008[5]	Prospective, non-randomized controlled	Italy	Severe PAD (Leriche-Fontaine III or IV)
Gu et al, Chin Med J 2008[33]	Prospective, randomized	China	CLI (including 1 TAO)
Napoli et al, Eur J Cardio Prev Rehab 2008[6]	Prospective, non-randomized controlled	Italy	Advanced PAD (Fontaine III or IV)
Intramuscular BM-MNC Implantation			
Gu et al, Chin Med J 2008[33]	Prospective, randomized	China	CLI (including 1 TAO)
Matoba et al, Am Heart J 2008[21]	Multi-institutional follow-up study to Tateishi et al 2002	Japan	PAD (Fontaine II or IV) and TAO
Motukuru et al, J Vasc Surg 2008[31]	Prospective, case series	India	TAO
Van Tongeren et al, J Cardiovasc Surg 2008[32]	Prospective, non-randomized	Netherlands	PAD (no options for revascularization) (CLI or claudication)
Wester et al, Scand J Surg 2008[22]	Prospective, case series	Norway	CLI (Rutherford II or III)
De Vriese et al, J Int Med 2007[27]	Prospective, case series	Belgium	ASO (Rutherford Cat 4-6)
Hernandez et al, Atherosclerosis 2007[29]	Prospective, pilot	Cuba	Severe PAD/CLI
Kajiguchi et al, Circ J 2007[9]	Prospective, case series	Japan	ASO and TAO
Saito et al, Circ J 2007[36]	Prospective, case series	Japan	TAO (Fontaine III or IV)
Miyamoto et al, Circulation 2006[30]	Prospective, case series, pilot	Japan	TAO
Nizankowski et al, Kardiol Pol 2005[34]	Prospective, case series	Poland	ASO and TAO
Higashi et al, Circulation 2004[12]	Prospective, case series	Japan	PAD
Saigawa et al, Circ J 2004[35]	Prospective, case series	Japan	ASO (Fontaine IIB-IV)
Esato et al, Cell Transplant 2002[11]	Prospective, case series	Japan	ASO and TAO
Tateishi et al, Lancet 2002[14]	Pilot, controlled	Japan	CLI
Tateishi et al, Lancet 2002[14]	Prospective, randomized, controlled	Japan	CLI
Intramuscular and intermetatarsal BM-MNC implantation			
Durdu et al, J Vasc Surg 2006[28]	Prospective consecutive series	Turkey	TAO (Rutherford II or III)

ASO, Atherosclerosis obliterans; *BM-MNC,* bone marrow mononuclear cell; *CLI,* chronic or critical limb ischemia; *PAD,* peripheral arterial disease; *TAO,* thromboangiitis obliterans or Buerger's disease.

SD = 0.10, median = 0.07, range = −0.03-0.22) arteries, the differences compared with the pre-implantation measurements were not statistically significant.

After BM-MNC implantation, two (22.2%) patients required minor amputations and three (33.3%) patients required major amputations of the involved limb due to continuing osteomyelitis or worsening gangrene. In two of the five cases, improvements in ABI measurements were observed after BM-MNC implantation. All amputation sites healed. The two minor amputations both were trans-

Table VI. Continued.

BM-MNC treated patients	*Gender*	*Mean age (years)*
9 (9 limbs)	5 (55.3%) females 4 (44.4%) males	61.7 (r, 43.0-85.0)
12 (12 limbs) (8 CLI; 3 claudication; 1 NA)	3 (25.0%) females 9 (75.0%) males	66.9
13 (13 limbs)	3 (23.1%) females 10 (76.9%) males	69.0
24 (28 limbs)	11 (45.8%) females 13 (54.2%) males	68.0 (median) (r, 26.0-85.0)
10 (10 limbs)	4 (40.0%) females 6 (60.0%) males	60.3 (r, 38.0-77.0)
16 (17 limbs)	12 (37.5%) females 20 (62.5%) males (entire group)	69.5 (r, 51.0-82.0) (entire group)
18 (18 limbs)	7 (38.9%) females 11 (61.1%) males	71.6 (r, 43.0-90.0)
16 (18 limbs)	12 (37.5%) females 20 (62.5%) males (entire group)	69.5 (r, 51.0-82.0) (entire group)
115 (115 limbs) (74 PAD; 41 TAO)	25 (21.7%) females 90 (78.3%) males	67 (r, 37-82) PAD Group 49 (r, 29-72) TAO Group
38 enrolled (38 limbs) (36 treated)	0 (0.0%) females 38 (100.0%) males	34.0
15 (15 limbs) (11 CLI; 3 claudication; 1 NA)	5 (33.3%) females 10 (66.7%) males	69.8
8 (8 limbs) 3 Rutherford II (all Cat 4); 5 Rutherford III (4 Cat 5; 1 Cat 6)	4 (50.0%) females 4 (50.0%) males	71.1 (r, 43.0-81.0)
16 (16 limbs)	8 (50.0%) females 8 (50.0%) males	78.0
12 (12 limbs)	4 (33.3%) females 8 (66.7%) males	62.7
7 (7 limbs) (4 ASO cases; 3 TAO)	2 (28.6%) females 5 (71.4%) males	53.4 (r, 37.0-67.0)
9 (9 limbs)	1 (11.1%) female 8 (88.9%) males	43.7 (r, 29.0-55.0)
7 (9 limbs)	1 (14.3%) female 6 (85.7%) males	47.9 (r, 28.0-63.0)
10 (10 limbs) (3 ASO; 7 TAO)	4 (40.0%) females 6 (60.0%) males	48.7 (r, 37.0-77.0)
7 (7 limbs)	1 (14.3%) female 6 (85.7%) males	64.0 (r, 51.0-74.0)
8 (8 limbs)	1 (12.5%) female 7 (87.5%) males	62.4 (r, 48.0-79.0)
8 (8 limbs) (4 ASO; 4 TAO)	NA	70.4 (r, 55.0-78.0)
29 enrolled (29 limbs) (25 treated)	5 (20.0%) females 20 (80.0%) males	66.0
23 enrolled (23 limbs) (22 treated, 20 in analysis)	2 (10.0%) females 18 (90.0%) males (analysis group)	69.0 (analysis group)
28 (28 limbs)	3 (10.7%) females 25 (89.3%) males	42.6 (r, 25.0-56.0)

metatarsal amputations, which were more limited amputations than would have been performed had the patients not undergone BM-MNC implantation. The three major amputations were performed between 4.1 to 11.0 weeks after BM-MNC implantation. Prior to the procedure, these three patients had Rutherford Grade IV (1 Category 5; 2 Category 6) and Fontaine IV classifications. One (11.1%) patient required a below-knee amputation (BKA), whereas

Table VII. Comparison of co-morbidities and ulceration status between previously published studies utilizing BM-MNC for the treatment of lower extremity ischemia

	Smoking/nicotine use (%)	*Diabetes (%)*	*Hypertension (%)*
Intra-arterial and intramuscular BM-MNC implantation			
Current study 2009	88.9%	77.8%	100.0%
Van Tongeren et al, J Cardiovasc Surg 2008[32]	83.3% (10 of 12 pts)	25.0% (3 of 12 pts)	41.7% (5 of 12 pts)
Bartsch T et al, Clin Res Cardiol 2007[15]	38.5% (5 of 13 pts)	38.5% (5 of 13 pts)	100.0%
Intra-arterial BM-MNC implantation			
Chochola et al, Int Angiol 2008[26]	33.3% (8 of 24 pts)	62.5% (15 of 24 pts)	75.0% (18 of 24 pts)
Cobellis et al, Bone Marrow Transplant 2008[5]	50.0% (5 of 10 pts)	70.0% (7 of 10 pts)	70.0% (7 of 10 pts)
Gu et al, Chin Med J 2008[33]	NA	90.6% (29 of 32 pts) (entire group)	NA
Napoli et al, Eur J Cardio Prev Rehab 2008[6]	66.7% (12 of 18 pts)	50.0% (9 of 18 pts)	61.1% (11 of 18 pts)
Intramuscular BM-MNC implantation			
Gu et al, Chin Med J 2008[33]	NA	90.6% (29 of 32 pts) (entire group)	NA
Matoba et al, Am Heart J 2008[21]	72.2% (83 of 115 pts)	47.8% (55 of 115 pts)	57.4% (66 of 115 pts)
Motukuru et al, J Vasc Surg 2008[31]	100.0%	0.0%	NA
Van Tongeren et al, J Cardiovasc Surg 2008[32]	86.7% (13 of 15 pts)	33.3% (5 of 15 pts)	46.7% (7 of 15 pts)
Wester et al, Scand J Surg 2008[22]	NA	0.0%	50.0% (4 of 8 pts)
De Vriese et al, J Int Med 2007[27]	31.0%	44.0%	88.0%
Hernandez et al, Atherosclerosis 2007[29]	58.3% (7 of 12 pts)	75.0% (9 of 12 pts)	66.7% (8 of 12 pts)
Kajiguchi et al, Circ J 2007[9]	57.1% (4 of 7 pts)	14.3% (1 of 7 pts)	14.3% (1 of 7 pts)
Saito et al, Circ J 2007[36]	NA	22.2%* (2 of 9 pts)	11.1% (1 of 9 pts)
Miyamoto et al, Circulation 2006[30]	85.7% (6 of 7 pts)	14.3% (1 of 7 pts)	42.9% (3 of 7 pts)
Nizankowski et al, Kardiol Pol 2005[34]	NA	0.0%	30.0% (3 of 10 pts)
Higashi et al, Circulation 2004[12]	100.0%	0.0%	42.9% (3 of 7 pts)
Saigawa et al, Circ J 2004[35]	NA	75.0% (6 of 8 pts)	100.0% (8 of 8 pts)
Esato et al, Cell Transplant 2002[11]	NA	NA	NA
Tateishi et al, Lancet 2002[14]	NA	72.0% (18 of 25 pts)	72.0% (18 of 25 pts)
Tateishi et al, Lancet 2002[14]	NA	65.0% (13 of 20 pts) (analysis group)	70.0% (14 of 20 pts) (analysis group)
Intramuscular and intermetatarsal BM-MNC implantation			
Durdu et al, J Vasc Surg 2006[28]	100.0%	0.0%	10.7% (3 of 28 pts)

ABI, Ankle brachial index; *BM-MNC,* bone marrow mononuclear cell.
*Instead of reporting prevalence of diabetes, Saito Y et al reported prevalence of impaired glucose tolerance.

the other two (22.2%) patients required above-knee amputations (AKAs). One patient requiring an AKA had a nonviable limb and was facing a probable hip disarticulation, as evidenced by the absence of anatomically named blood vessels to the thigh on angiogram, with only collaterals from the right internal iliac artery. Stem cell therapy was performed in an effort to re-establish circulation, which would possibly enable performing an AKA rather than a hip disarticulation. No improvement was observed between the ABI measurements before and after BM-MNC implantation, as ankle measurements were undetectable at both evaluations. However, the thigh was warm on physical examination, indicating possible improved collateral circulation. Because the muscle was viable at the time of surgery, an AKA was performed.

Major amputation was avoided in six (66.7%) of the nine patients undergoing BM-MNC implantation. Five of these six patients did not have rest pain at the three-month follow-up evaluation, while rest pain could not be assessed in the aforementioned patient with hemiparesis. All six patients exhibited as a Fontaine classification of II, which was an improvement by at least one level from the preprocedure evaluation. Five (83.3%) of six patients had Rutherford classifications of I3 (severe claudication), corresponding to an improvement by at least one grade compared with baseline assessments. In addition, complete wound healing was achieved within three months in these three patients who had ulcers prior to BM-MNC implantation and for whom amputation was not required, including in one patient who had a gangrenous foot ulcer. Two of these patients had foot ulcers; the other patient had an ulcer on the anterior tibia. At a mean follow-up of 7.8 months (median = 7.1; SD = 5.9; range, 0.50-16.8 months), no amputations have been required for these six patients.

Outcomes also were assessed using the four-pronged definition of success (Table V). According to this definition, BM-MNC implantation was successful in three (33.3%) patients, as major amputation was avoided and the other applicable criteria were met. Two (22.2%) additional patients met three of the four criteria for success, including

Table VII. Continued.

Hypercholesterolemia/ hyperlipidemia (%)	*Non-healing ulcers (%)*	*Gangrene (%)*	*Mean pre-BM-MNC ABI*
55.6%	88.9% (8 of 9 pts)	33.3% (3 of 9 pts)	Dorsalis pedis = 0.21 (r, 0.00-0.50) Posterior tibial = 0.26 (r, 0.00-0.48)
33.3% (4 of 12 pts)	NA	NA	NA
100.0%	NA	NA	0.66
54.2% (13 of 24 pts)	58.3% (14 of 24 pts)	NA	0.57 (0.00-1.00)
NA	NA	NA	0.75 (r, 0.31-1.16)
NA	NA	NA	NA
NA	44.4% (8 of 18 pts)	NA	0.41 (r, 0.20-0.75)
NA	NA	NA	NA
16.5% (19 of 115 pts)	NA	NA	NA
NA	100.0%	NA	0.26 (r, 0.12-0.47)
26.7% (4 of 15 pts)	NA	NA	NA
NA	62.5% (5 of 8 pts)	NA	NA
69.0%	76.0% 38.0% w/ gangrene 38.0% w/o gangrene	38.0%	0.40
NA	41.7% (5 of 12 pts)	41.7% (5 of 12 pts)	0.30-0.32 (median)
28.6% (2 of 7 pts)	42.9% (3 of 7 pts)	NA	0.52 (r, 0.00-1.09)
22.2% (2 of 9 pts)	88.9% (8 of 9 pts)	NA	NA
14.3% (1 of 7 pts)	100.0%	NA	0.71 (r, 0.34-1.10)
NA	60.0% (6 of 10 pts)	40.0% (4 of 10 pts)	0.39
57.1% (4 of 7 pts)	100.0%	NA	0.38 (r, 0.19-0.60)
62.5% (5 of 8 pts)	NA	NA	0.54 (r, 0.00-1.32)
NA	37.5% (3 of 8 pts)	NA	NA
32.0% (8 of 25 pts)	24.0% (6 of 25 pts)	32.0% (8 of 25 pts)	0.34
55.0% (11 of 20 pts) (analysis group)	20.0% (4 of 20 pts) (analysis group)	50.0% (10 of 20 pts) (analysis group)	0.37 (analysis group)
10.7% (3 of 28 pts)	64.3% (18 of 28 pts)	NA	0.52

not undergoing a major amputation. One (11.1%) patient did not require a major amputation and no longer had rest pain. The final (11.1%) patient did not meet any of the four criteria for success. This patient was the aforementioned 50-year-old male for whom BM-MNC implantation was performed to avoid a probable hip disarticulation.

DISCUSSION

Treatment options for patients with severe PAD for whom conservative management has failed and who are not candidates for surgical interventions, such as endovascular or open procedures, are sparse. Such patients typically must resort to limb amputation as a final option. Beginning in 2000, several animal model studies reported successful outcomes using stem cell therapy to improve peripheral blood circulation.[10,13,17,19,20,25] These studies subsequently spurred early clinical trials in Asia and Europe involving injection of mononuclear bone marrow stem cells to treat severe PAD.[3,5,6,9,11,12,14,15,21,22,26-32] Results from these preliminary trials were positive, leading investigators to recommend further study examining the efficacy of stem cell therapy for PAD.[6,9,11,12,14,22,26,27,29,31,33-36] The purpose of the current study was to present short-term results of dual intramuscular and intra-arterial autologous BM-MNC implantation for the treatment of patients with severe PAD for whom amputation was recommended. To our knowledge, it is the first report of this type of stem cell therapy for PAD from investigators in the United States.

The goal of this type of stem cell therapy is to promote neoangiogenesis, thereby increasing circulation, reducing symptoms, and facilitating wound healing in patients with PAD by injecting autologous mononuclear cells obtained from bone marrow of the iliac crest into various low-oxygenated ischemic sites of the lower extremity. After stem cell isolation, no pre-selection or cell sorting was conducted. Mononuclear cell types including hematopoetic cells, angioblasts, and mesenchymal cells, were transplanted to allow multiple means of regeneration.[15] Similar to the technique described by Bartsch et al, both intra-arterial and intramuscular injections were used in the cur-

Table VIII. Comparison of outcomes between previously published studies utilizing BM-MNC for the treatment of lower extremity ischemia

	Study definition of success or primary endpoints (follow-up)	*Mean post-BM-MNC ABI*
Current study, 2009	Success definition (3 mos) 1) Improvement of ABI; 2) Absence of rest pain; 3) Ulcer healing, if applicable; and 4) No major amputations	Dorsalis pedis = 0.34 (r, 0.00-0.74) Posterior tibial = 0.31 (r, 0.00-0.69)
Other studies using intra-arterial and intramuscular BM-MNC implantation		
Van Tongeren et al, J Cardiovasc Surg 2008[32]	Success definition (1 mos/6 mos/12 mos): 1) Full wound recovery; and/or 2) Doubling of walking distance.	NA
Bartsch et al, Clin Res Cardiol 2007[15]	Primary endpoints (2 mos/13 mos): 1) Maximum walking distance; 2) ABI (at rest/after treadmill test); 3) Venous occlusion plethysmography; and 4) Thigh and lower leg capillary-venous oxygen saturation	0.80/0.80 (2 mos/13 mos)
Other studies including severe PAD		
Higashi et al, Circulation 2004[12]	NA (1 mos/6 mos)	0.39 (r, 0.22-0.62) at 4 wks 0.35 (r, 0.21-0.59) at 24 wks
Tateishi et al, Lancet 2002[14]	Success definition (1 mos/6 mos) improvement in: 1) ABI > 0.1 2) Transcutaneous oxygen pressure; and 3) Rest pain	0.47 at 4 wks
Tateishi et al, Lancet 2002[14]	Success definition (1 mos/6 mos) improvement in: 1) ABI > 0.1 2) Transcutaneous oxygen pressure; and 3) Rest pain	0.46 at 4 wks

ABI, Ankle brachial index; *BM-MNC,* bone marrow mononuclear cell.

rent study to maximize the local concentration of stem cells in the ischemic area and increase cell invasion.[15] The intra-arterial injection portion of this combined technique ensures that the stem cells reach all targeted vessels in an antegrade manner, allowing ischemic muscle regions that still are perfused to receive a high concentration of stem cells.[15] The intramuscular injection enables ischemic musculature to receive a high concentration of stem cells through several local sites, which results in local dispersion into the surrounding muscle.[15] Because most patients with advanced PAD have multi-level disease, including extensive pathology of the femoropopliteal tract and pedal arteries, the exclusive use of intramuscular injections, especially when limited to the calf, theoretically may not affect these regions, which may be accessed by intra-arterial injections.[32] Conversely, local intramuscular injections may be of particular importance for those patients who have superficial femoral artery occlusions that would prevent stem cell injections intra-arterially from reaching ischemic muscles.[15]

No procedure-related complications were reported during the technique or at the follow-up evaluations. Mean improvements of 0.13 and 0.09 in ABI were observed after the BM-MNC implantation for the dorsalis pedis and posterior tibial ankle arteries, respectively. Major amputation was avoided in six (66.7%) of the nine patients undergoing BM-MNC implantation through a mean follow-up of 7.8 months. These six patients also showed improvement in symptom severity three months after the procedure, as evidenced by alleviation of rest pain and improvements by at least one level in Rutherford and Fontaine classifications. In addition, complete wound healing was achieved within three months in all three patients who had ulcers prior to BM-MNC implantation and for whom amputation was not required. This specific BM-MNC implantation technique was fully successful in three (33.3%) patients, as major amputation was avoided and other applicable criteria were met. Five (55.6%) additional patients demonstrated success in at least one of the four criteria. The three (33.3%) patients who underwent major amputation all exhibited Rutherford III and Fontaine IV classifications, which are the most severe chronic limb ischemia and PAD classifications. One patient underwent a BKA 4.1 weeks after the BM-MNC implantation and two patients underwent AKAs at 5.4 and 11.0 weeks after the procedure. Because this study population was comprised of limb salvage patients, any improvement in PAD and postponement of major amputation should be considered successful. Furthermore, after BM-MNC implantation, sufficient blood flow was established so that three of the five patients requiring post-implantation amputations received more limited am-

Table VIII. Continued.

Absence of rest pain (%)	*Complete ulcer healing (%)*	*Major amputation (%)*	*Success rate per study definition or endpoint results*
100.0% (5 of 5 pts)	62.5% (5 of 8 pts)	33.3% (3 of 9 pts)	33.3% (3 of 9 pts)
NA	NA	16.7% (2 of 12 pts)	66.7% (8 of 12 pts)
NA	NA	NA	(2 mos/13 mos, all stat sign) 1) Mean = 500 m/542 m; 2) Rest mean = 0.80/0.80, Stress mean = 0.76/0.75; 3) Rest mean = 2.5/2.6, Reactive hyperemia Mean = 7.2/6.8; 4) Thigh mean = 66/67, Lower leg mean = 63/66
100.0% (Pain-free walking)	NA	0.0%	NA
48.0% (12 of 25 pts)	50.0% (3 of 6 pts)	0.00%	1) 68.0% (17 of 25 pts) 2) 100% (25 of 25 pts) 3) 48.0% (12 of 25 pts)
50.0% (10 of 20 pts)	75.0% (3 of 4 pts)	0.00%	1) 65.0% (13 of 20 pts) 2) 100% (20 of 20 pts) 3) 50.0% (10 of 20 pts)

putations than would have been performed had the patients not participated in the study.

Comparison of outcomes between lower extremity stem cell therapy studies is difficult, as several different injection techniques were used, indications for stem cell therapy varied, and differences in patient populations exist (Tables VI and VII). Several single-injection site studies involving intra-arterial injections[5,6,26,33] and many studies utilizing intramuscular injections[9,11,12,14,21,22,27,29-36] have shown promising results. In addition, the two other groups using combined intra-arterial and intramuscular injections also reported successful outcomes.[3,15,32] Bartsch et al also had a novel addition to their technique involving ischemic preconditioning by incorporating exercise prior to injection and using a blood pressure cuff as a tourniquet to compress the thigh for a few minutes after the intra-arterial injection, thereby creating a stop flow in the leg to extend contact between the stem cells and ischemic tissue.[3,15] Promising results also have been reported using a combined intramuscular and intermetatarsal injection technique.[28]

The majority of lower extremity stem cell therapy studies include patients with mild forms of PAD (ABI $\geq$ 0.70)[5,6,26,32] or solely included patients with thromboangiitis obliterans (TAO, Buerger's disease) or atherosclerosis obliterans (ASO).[9,11,27,28,30,31,34-36] Only two previously published studies (both using intramuscular injection techniques) have included patients with severe PAD (mean ABI $<$ 0.40).[12,14]

Comparing these two studies with the current study, the study population sizes of the three studies ranged from seven to 25 patients (Table VI).[12,14] The current study was comprised of an approximately equal number of females and males, whereas the other two studies involved a disproportionately higher percentage of males (Table VI).[12,14] All three study populations involved patients with considerable co-morbidities; however, the Higashi et al study excluded patients with diabetes (Table VII).[12,14] The study by Tateishi et al had considerably fewer patients with nonhealing ulcers than the other two studies (Table VII).[12,14] The mean pre-treatment ABI measurements in each of the three studies corresponded to severe PAD (ABI $<$ 0.40) (Table VII).[12,14] Improvement in ABI measurements after BM-MNC implantation were reported for all three studies; however, the mean post-procedure ABI measurements remained in the severe PAD range in the Higashi et al and current studies, but corresponded to moderate PAD (ABI $<$ 0.70) in the Tateishi et al study (Table VIII).[12,14] All patients in the Higashi et al study had pain-free ambulation after the procedure, whereas no patients in the cur-

Table IX. A comparison of study population percentages with ABI improvement greater than 0.1 after lower extremity stem cell therapy

	BM-MNC implantation technique	*% of cases with ABI improvement > 0.1*
Intra-arterial and intramuscular BM-MNC implantation		
Current Study, 2009	Intra-arterial & Intramuscular	55.6% (5 of 9)
Intra-arterial BM-MNC implantation		
Cobellis et al, Bone Marrow Transplant 2008[5]	Intra-arterial	40.0% (4 of 10)
Gu et al, Chin Med J 2008[33]	Intra-arterial	41.2% (7 of 17)
Napoli et al, Eur J Cardio Prev Rehab 2008[6]	Intra-arterial	55.6% (10 of 18)
Intramuscular BM-MNC implantation		
Gu et al, Chin Med J 2008[33]	Intramuscular	44.4% (8 of 18)
Motukuru et al, J Vasc Surg 2008[31]	Intramuscular	91.7% (33 of 36)
Hernandez et al, Atherosclerosis 2007[29]	Intramuscular	64% NA
Kajiguchi et al, Circ J 2007[9]	Intramuscular	42.9% (3 of 7)
Miyamoto et al, Circulation 2006[30]	Intramuscular	28.6% (2 of 7)
Esato et al, Cell Transplant 2002[11]	Intramuscular	12.5% (1 of 8)
Tateishi et al, Lancet 2002[14]	Intramuscular	66.7% (30 of 45)

ABI, Ankle brachial index; *BM-MNC,* bone marrow mononuclear cell.

rent study and approximately half of the patients in the Tateishi et al study did not have rest pain (Table VIII).[12,14] The majority of ulcers healed in both the current study and that by Tateishi et al (Table VIII).[12,14] Finally, major amputations were performed in three patients in the current study, but were not required in the other two studies.[12,14]

The three studies that examined the dual intra-arterial and intramuscular injection technique, including the current one, utilized the procedure in populations of less than 15 patients (Table VI).[15,32] The other two studies had male:female gender ratios of approximately 3:1, compared with the nearly equivalent gender distribution in the current study (Table VI).[15,32] The current study involved patients with considerably more co-morbidities than the previous dual injection studies and, unlike the other studies, included patients with non-healing ulcers (Table VII).[15,32] The Van Tongeren et al study included patients with mild PAD (ABI $\geq$ 0.70).[32] The mean pre-treatment dorsalis pedis and posterior tibial ABI measurements of 0.21 and 0.26, respectively, in the current study corresponded to severe PAD (ABI $<$ 0.40), compared with the mean ABI of 0.66 in the Bartsch et al study that corresponded to moderate ABI (ABI $<$ 0.7) (Table VII).[15] Both studies demonstrated improvement in ABI after the procedure, with the dorsalis pedis and posterior tibial ABI measurements of 0.34 and 0.31, respectively, in the current study remaining in the severe PAD range, compared with the mean ABI of 0.80 in the Bartsch et al study that corresponded to mild PAD (Table VIII).[15,32] Two (16.7%) patients in the Van Tongeren et al and three (33.3%) patients in the current study required major amputations after the procedure (Table VIII).[15,32]

Finally, in an effort to compare results between all of the published lower extremity stem cell studies, the percentage of patients experiencing an increase of more than 0.1 in ABI measurements after stem cell implantation is presented in Table IX. Using this widely accepted definition of vascular function improvement, which was first proposed by Rutherford et al and also was used in the seminal stem cell study by Tateishi et al, five (45.5%) of 11 studies, including the current study, demonstrated improvement in the majority of patients.[6,14,24,29,31]

The minimally invasive nature of the injection procedure itself and the use of autologous cells make stem cell therapy an attractive option for managing a wide range of ischemic conditions, as demonstrated by previous studies and confirmed by the current study. Besides lending further support to the growing list of studies recommending stem cell therapy for the management of PAD, the successful results of the current study also add several new findings. First, this study is only the third report involving dual intramuscular and intra-arterial injection and the first utilizing the dual technique in more complex cases, including in the presence of non-healing ulcers. Because all three studies documented improvement, attempting to promote neoangiogenesis through multiple physiological pathways appears beneficial and warrants further study. Second, unlike previous studies, most of which used stem cell therapy to treat earlier stages of PAD, the current study utilized BM-MNC implantation as a means of limb salvage therapy. Additional alternatives are desperately needed for patients with end-stage PAD who currently have few management options available other than amputation. Overall survival after BKA and AKA has been reported to be 70% and 35% at one and five years, respectively.[37] Therefore, avoiding or even postponing amputation has a significant impact on patient quality of life and survival. Third, the study population in this study is more heterogeneous than those in previous studies and, therefore, may better represent the general PAD patient population as a whole. In addition, since BM-MNC implantation was utilized in patients with complex medical conditions, including severe PAD, the presence of significant co-morbidities, and open ulcers in all but one case, these "worse case" results suggest that this stem cell therapy procedure may be beneficial not only to

limb salvage patients, but also in patients with earlier stages of the disease. Many studies also either entirely excluded patients with diabetes or those with poorly controlled or uncontrolled diabetes, whereas 77.8% of patients in the current study had diabetes.[6,12,14,22,31,34,36] Again, the results of studies with strict inclusion criteria or those limited to conditions such as ASO or TAO, which affect very specific patient populations, may not accurately depict the results that may be expected in the typical PAD patient with diabetes.

Study limitations include the small population size, lack of a control group, and absence of long-term follow-up. A prospective control group was not feasible, as the senior investigator (R.W.F.) did not wish to withhold this promising treatment from patients who were at end stages of the disease and for whom amputation was the only alternative. Another challenge for all stem cell studies is demonstrating proof of neoangiogenesis, as visual demonstration is difficult due to inherent variability in technique and diagnostic imaging protocols. In light of this difficulty and because this particular study population involved patients with end-stage PAD who had significant co-morbidities, the senior author chose not to place patients at unnecessary risk by including follow-up angiography as part of the study protocol. The patients who did not undergo major amputation after BM-MNC implantation will continue to be followed up to establish a long-term prognosis of the technique and to assist in shaping surgeon and patient expectations of the procedure.

Because the research involving BM-MNC implantation is in its infancy, many questions remain. Histological analysis of amputated specimens would assist in determining the cause of tissue loss and locating possible areas of robust neovascular response to verify neoangiogenesis. Endothelial surface marker testing or tissue mitogen assays also would assist in understanding the physiological response to BM-MNC implantation. Additional study involving the use of BM-MNC implantation as a limb salvage technique is crucial so that patients in end stages of PAD may be provided with alternatives to avoid or postpone amputation. General multi-center prospective stem cell therapy studies for the management of PAD would be beneficial so that its efficacy could be examined using this standardized technique and in a larger patient population. The ideal number of intramuscular injection sites also has not been elucidated, with techniques ranging from one to 90 sites reported in the literature.[9,11,12,14,15,21,22,27-36] Finally, as recommended for other PAD management procedures, standards for evaluation and reporting the results of stem cell therapies are critical to compare results between studies.

In conclusion, eight (88.9%) of nine patients showed some level of clinical improvement after BM-MNC implantation, suggesting that the therapeutic goal of improving limb perfusion was achieved. In three cases, more limited amputations were performed than would have been required had the patients not undergone BM-MNC implantation. Three patients with the most severe classifications of chronic ischemia and PAD possible required major amputations, but amputation was postponed one to three months. All amputation sites healed following surgery. Major amputation was avoided in six (66.7%) patients, who have not required amputation at a mean follow-up of 7.8 months. Complete wound healing was achieved within three months in all patients who had ulcers prior to BM-MNC implantation and for whom amputation was not required. These short-term results indicate the use of BM-MNC implantation as a means of limb salvage therapy for patients with severe PAD shows promise in postponing or avoiding amputation in a patient population currently presented with few alternatives to amputation. Furthermore, in light of these encouraging results achieved in "worst-case" scenarios and based on results in the literature, the authors plan to perform the procedure earlier in the treatment plan of patients with severe ischemia or non-healing ulcers. Future study also is warranted to determine if patients with moderate PAD may also benefit from this procedure.

AUTHOR CONTRIBUTIONS

Conception and design: RF, AP, KS, TH
Analysis and interpretation: RF, AP, JH, MW
Data collection: RF, AP, JH, MW
Writing the article: RF, AP, JH, MW
Critical revision of the article: RF, AP, KS, TH, JH, MW
Final approval of the article: RF, AP, KS, TH, JH, MW
Statistical analysis: RF, JH, MW
Obtained funding: N/A
Overall responsibility: RF

REFERENCES

1. Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA 2001;286:1317-24.
2. Tachi Y, Fukui D, Wada Y, Koshikawa M, Shimodaira S, Ikeda U, et al. Changes in angiogenesis-related factors in serum following autologous bone marrow cell implantation for severe limb ischemia. Expert Opin Biol Ther 2008;8:705-12.
3. Bartsch T, Brehm M, Zeus T, Strauer BE. Autologous mononuclear stem cell transplantation in patients with peripheral occlusive arterial disease. J Cardio Nurs 2006;21:430-2.
4. Hooi JD, Kester AD, Stoffers HE, Overdijk MM, van Ree JW, Knottnerus JA. Incidence of and risk factors for asymptomatic peripheral arterial occlusive disease: a longitudinal study. Am J Epidemiol 2001; 153:666-72.
5. Cobellis G, Silvestroni A, Lillo S, Sica G, Botti C, Maione C, et al. Long-term effects of repeated autologous transplantation of bone marrow cells in patients affected by peripheral arterial disease. Bone Marrow Transplant 2008;42:667-72.
6. Napoli C, Farzati B, Sica V, Iannuzzi E, Coppola G, Silvestroni A, et al. Beneficial effects of autologous bone marrow cell infusion and antioxidants/L-arginine in patients with chronic critical limb ischemia. Eur J Cardiol Prev Rehab 2008;15:709-18.
7. Dillingham TR, Pezzin LE, Mackenzie EJ. Limb amputation and limb deficiency: epidemiology and recent trends in the United States. South Med J 2002;95:875-83.
8. Goldman R, Rosen M, Brewley B, Golden M. Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. Adv Skin Wound Care 2004;17:284-94.
9. Kajiguchi M, Kondo T, Izawa H, Kobayashi M, Yamamoto K, Shintani S, et al. Safety and efficacy of autologous progenitor cell transplantation

for therapeutic angiogenesis in patients with critical limb ischemia. Circ J 2007;71:196-201.
10. Hamano K, Li TS, Kobayashi T, Tanaka N, Kobayashi S, Matsuzaki M, et al. The induction of angiogenesis by the implantation of autologous bone marrow cells: a novel and simple therapeutic method. Surgery 2001;130:44-54.
11. Esato K, Hamano K, Li TS, Furutani A, Seyama A, Takenaka H, et al. Neovascularization induced by autologous bone marrow cell implantation in peripheral arterial disease. Cell Transplant 2002;11:747-52.
12. Higashi Y, Kimura M, Hara K, Noma K, Jitsuiki D, Nakagawa K, et al. Autologous bone-marrow mononuclear cell implantation improves endothelium-dependent vasodilation in patients with limb ischemia. Circulation 2004;109:1215-8.
13. Iwase T, Nagaya N, Fujii T, Itoh T, Ishibashi-Ueda H, Yamagishi M, et al. Adrenomedullin enhances angiogenic potency of bone marrow transplantation in a rat model of hindlimb ischemia. Circulation 2005; 111:356-62.
14. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 2002;360:427-35.
15. Bartsch T, Brehm M, Zeus T, Kogler G, Wernet P, Strauer BE. Transplantation of autologous mononuclear bone marrow stem cells in patients with peripheral arterial disease (The TAM-PAD study). Clin Res Cardiol 2007;96:891-9.
16. Hattori R, Matsubara H. Therapeutic angiogenesis for severe ischemic heart diseases by autologous bone marrow cells transplantation. Mol Cell Biochem 2004;264:151-5.
17. Ikenaga S, Hamano K, Nishida M, Kobayashi T, Li TS, Kobayashi S, et al. Autologous bone marrow implantation induced angiogenesis and improved deteriorated exercise capacity in a rat ischemic hindlimb model. J Surg Res 2001;96:277-83.
18. Ishida A, Ohya Y, Sakuda H, Ohshiro K, Higashiuesato Y, Nakaema M, et al. Autologous peripheral blood mononuclear cell implantation for patients with peripheral arterial disease improves limb ischemia. Circ J 2005;69:1260-5.
19. Isner JM, Asahara T. Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. J Clin Invest 1999;103: 1231-6.
20. Kobayashi T, Hamano K, Li TS, Katoh T, Kobayashi S, Matsuzaki M, et al. Enhancement of angiogenesis by the implantation of self bone marrow cells in a rat ischemic heart model. J Surg Res 2000;89:189-95.
21. Matoba S, Tatsumi T, Murohara T, Imaizumi T, Katsuda Y, Ito M, et al. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. Am Heart J 2008;156:1010-8.
22. Wester T, Jorgensen JJ, Stranden E, Sandbaek G, Tjonnfjord G, Bay D, et al. Treatment with autologous bone marrow mononuclear cells in patients with critical lower limb ischaemia. A pilot study. Scand J Surg 2008;97:56-62.
23. Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, Ahn S, et al. Recommended standards for reports dealing with lower extremity ischemia: Revised version. J Vasc Surg 1997;26:517-38.
24. Rutherford RB, Flanigan DP, Gupta SK, Johnston KW, Karmody A, Whittemore AD, et al. Suggested standards for reports dealing with lower extremity ischemia. J Vasc Surg 1986;4:80-94.
25. Zhang H, Zhang N, Li M, Feng H, Jin W, Zhao H, et al. Therapeutic angiogenesis of bone marrow mononuclear cells (MNCs) and peripheral blood MNCs: transplantation for ischemic hindlimb. Ann Vasc Surg 2008;22:238-47.
26. Chochola M, Pytlik R, Kobylka P, Skalicka L, Kideryova L, Beran S, et al. Autologous intra-arterial infusion of bone marrow mononuclear cells in patients with critical leg ischemia. Int Angiol 2008;27:281-90.
27. De Vriese AS, Billiet J, Van Droogenbroeck J, Ghekiere J, De Letter JA. Autologous transplantation of bone marrow mononuclear cells for limb ischemia in a caucasian population with atherosclerosis obliterans. J Intern Med 2008;263:395-403.
28. Durdu S, Akar AR, Arat M, Sancak T, Eren NT, Ozyurda U. Autologous bone-marrow mononuclear cell implantation for patients with Rutherford grade II-III thromboangiitis obliterans. J Vasc Surg 2006; 44:732-9.
29. Hernandez P, Cortina L, Artaza H, Pol N, Lam RM, Dorticos E, et al. Autologous bone-marrow mononuclear cell implantation in patients with severe lower limb ischaemia: a comparison of using blood cell separator and Ficoll density gradient centrifugation. Atherosclerosis 2007;194:e52-6.
30. Miyamoto K, Nishigami K, Nagaya N, Akutsu K, Chiku M, Kamei M, et al. Unblinded pilot study of autologous transplantation of bone marrow mononuclear cells in patients with thromboangiitis obliterans. Circulation 2006;114:2679-84.
31. Motukuru V, Suresh KR, Vivekanand V, Raj S, Girija KR. Therapeutic angiogenesis in Buerger's disease (thromboangiitis obliterans) patients with critical limb ischemia by autologous transplantation of bone marrow mononuclear cells. J Vasc Surg 2008;48(6 Suppl):53S-60S.
32. Van Tongeren RB, Hamming JF, Fibbe WE, Van Weel V, Frerichs SJ, Stiggelbout AM, et al. Intramuscular or combined intramuscular/intra-arterial administration of bone marrow mononuclear cells: a clinical trial in patients with advanced limb ischemia. J Cardiovasc Surg 2008;49: 51-8.
33. Gu YQ, Zhang J, Guo LR, Qi LX, Zhang SW, Xu J, et al. Transplantation of autologous bone marrow mononuclear cells for patients with lower limb ischemia. Chin Med J 2008;121:963-7.
34. Nizankowski R, Petriczek T, Skotnicki A, Szczeklik A. The treatment of advanced chronic lower limb ischaemia with marrow stem cell autotransplantation. Kardiol Pol 2005;63:351-60.
35. Saigawa T, Kato K, Ozawa T, Toba K, Makiyama Y, Minagawa S, et al. Clinical application of bone marrow implantation in patients with arteriosclerosis obliterans, and the association between efficacy and the number of implanted bone marrow cells. Circ J 2004;68:1189-93.
36. Saito Y, Sasaki K, Katsuda Y, Murohara T, Takeshita Y, Okazaki T, et al. Effect of autologous bone-marrow cell transplantation on ischemic ulcer in patients with Buerger's disease. Circ J 2007;71:1187-92.
37. Aulivola B, Hile CN, Hamdan AD, Sheahan MG, Veraldi JR, Skillman JJ, et al. Major lower extremity amputation: outcome of a modern series. Arch Surg 2004;139:395-9.

Submitted Jun 11, 2009; accepted Jul 30, 2009.